

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. – 14. (Canceled).

15. (Currently Amended) A method for characterising a polypeptide, which method comprises the steps of:

(a) cleaving the polypeptide with a sequence specific cleavage reagent to form peptide fragments;

(b) capping one or more ϵ -amino groups that are present with a lysine reactive agent;

(c) analyzing the peptide fragments ~~according to the method of claim 1~~ by

(a) labelling the analyte with a light-absorbing label that absorbs light at a pre-determined frequency, to form a labelled analyte;

(b) embedding the labelled analyte in a matrix wherein the matrix comprises at least one light-absorbing compound, to form an embedded labelled analyte;

(c) desorbing the embedded labelled analyte by exposing it to light having the pre-determined frequency, to form a desorbed analyte; and

(d) detecting the desorbed analyte by mass spectrometry, to characterise the analyte to form a mass fingerprint for the polypeptide; and

(d) determining the identity of the polypeptide from the mass fingerprint.

16. (Currently Amended) A method for characterising a population of polypeptides, which method comprises the steps of:

- (a) separating one or more polypeptides from the population;
- (b) cleaving one or more polypeptides with a sequence specific cleavage reagent to form peptide fragments;
- (c) capping one or more ϵ -amino groups that are present with a lysine reactive agent;
- (d) analysing the peptide fragments ~~according to the method of claim 1 by~~
- (a) labelling the analyte with a light-absorbing label that absorbs light at a pre-determined frequency, to form a labelled analyte;
- (b) embedding the labelled analyte in a matrix wherein the matrix comprises at least one light-absorbing compound, to form an embedded labelled analyte;
- (c) desorbing the embedded labelled analyte by exposing it to light having the pre-determined frequency, to form a desorbed analyte; and
- (d) detecting the desorbed analyte by mass spectrometry, to characterise the analyte to form a mass fingerprint for one or more polypeptides; and
- (e) determining the identity of one or more polypeptides from the mass fingerprint.

17. (Currently Amended) A method for comparing a plurality of samples, each sample comprising one or more polypeptides, which method comprises the steps of:

- (a) separating one or more polypeptides from each of the samples;
- (b) cleaving the polypeptides with a sequence specific cleavage reagent to form peptide fragments;

- (c) capping one or more ϵ -amino groups that are present with a lysine reactive agent;
- (d) analysing peptide fragments ~~according to the method of claim 1 by~~
- (a) labelling the analyte with a light-absorbing label that absorbs light at a pre-determined frequency, to form a labelled analyte;
- (b) embedding the labelled analyte in a matrix wherein the matrix comprises at least one light-absorbing compound, to form an embedded labelled analyte;
- (c) desorbing the embedded labelled analyte by exposing it to light having the pre determined frequency, to form a desorbed analyte; and
- (d) detecting the desorbed analyte by mass spectrometry, to characterise the analyte to form a mass fingerprint for one or more polypeptides from the samples; and
- (e) determining the identity of one or more polypeptides in the samples from one or more mass fingerprints.

18. (Previously Presented) The method according to claim 15, wherein the lysine-reactive agent is a labelled lysine-reactive agent.

19. (Previously Presented) A method for comparing a plurality of samples, each sample comprising one or more polypeptides, which method comprises:

- (a) capping one or more ϵ -amino groups that are present in each sample with a labelled lysine reactive agent;
- (b) pooling the samples;
- (c) separating one or more polypeptides from the pooled samples;
- (d) cleaving the polypeptides with a sequence specific cleavage reagent to form peptide fragments;

- (e) analyzing peptide fragments by
- (a) labelling the analyte with a light-absorbing label that absorbs light at a pre-determined frequency, to form a labelled analyte;
- (b) embedding the labelled analyte in a matrix wherein the matrix comprises at least one light-absorbing compound, to form an embedded labelled analyte;
- (c) desorbing the embedded labelled analyte by exposing it to light having the pre determined frequency, to form a desorbed analyte; and
- (d) detecting the desorbed analyte by mass spectrometry, to characterise the analyte according to the method of claim 1 to form a mass fingerprint for one or more polypeptides from the samples; and
- (f) determining the identity of one or more polypeptides in the samples from one or more mass fingerprints;

wherein the same label is employed for polypeptides or peptides from the same sample, and different labels are employed for polypeptides or peptides from different samples, such that the sample from which a polypeptide or peptide originates can be determined from its label.

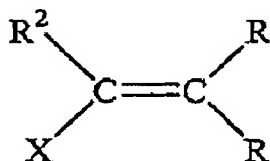
20. (Previously Presented) The method according to claim 19, wherein the sequence specific cleavage agent cleaves the one or more polypeptides on the C-terminal side of a lysine residue.

21. (Previously Presented) The method according to claim 19, wherein the specific cleavage agent comprises Lys-C or Trypsin.

22. (Previously Presented) The method according to claim 19, wherein the peptide fragments having capped ϵ -amino groups are removed by affinity capture, and wherein the lysine reactive agent comprises biotin.

23. (Previously Presented) The method according to claim 19, wherein the lysine reactive agent comprises a hindered Michael reagent.

24. (Previously Presented) The method according to claim 23, wherein the hindered Michael agent comprises a compound having the following structure:



wherein X is an electron withdrawing group that is capable of stabilizing a negative charge; the R groups independently comprise a hydrogen, a halogen, an alkyl, an aryl, or an aromatic group with the proviso that at least one of the R groups comprises a sterically hindering group; and the group R² comprises a hydrogen, a halogen, a hydrocarbon group, an electron withdrawing group, or a linker capable of attachment to an affinity capture functionality or a solid phase support.

25. – 50. (Canceled).

51. (Previously Presented) The method according to claim 15, further comprising reducing cysteine disulphide bridges in the polypeptide to form free thiols and capping the free thiols, prior to cleaving the polypeptide.

52. (Previously Presented) The method according to claim 15, further comprising deactivating the cleavage agent after cleaving the polypeptide.

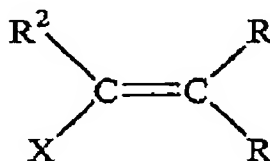
53. (Previously Presented) The method according to claim 15, wherein the sequence specific cleavage agent cleaves each isolated polypeptide on the C-terminal side of a lysine residue.

54. (Withdrawn) The method according to claim 15, wherein the sequence specific cleavage agent comprises Lys-C or Trypsin.

55. (Previously Presented) The method according to claim 15, wherein the peptide fragments having capped ϵ -amino groups are removed by affinity capture, and wherein the lysine reactive agent comprises biotin.

56. (Previously Presented) The method according to claim 15, wherein the lysine reactive agent comprises a hindered Michael reagent.

57. (Previously Presented) The method according to claim 56, wherein the hindered Michael agent comprises a compound having the following structure:



wherein X is an electron withdrawing group that is capable of stabilizing a negative charge; the r groups independently comprise a hydrogen, a halogen, an alkyl, an aryl, or an aromatic group with the proviso that at least one of the R groups comprises a sterically hindering

group; and the group R^2 comprises a hydrogen, a halogen, a hydrocarbon group, an electron withdrawing group, or a linker capable of attachment to an affinity capture functionality or a solid phase support.

58. (Previously Presented) The method according to claim 16, further comprising reducing cysteine disulphide bridges in the polypeptide to form free thiols and capping the free thiols, prior to cleaving the polypeptide.

59. (Previously Presented) The method according to claim 16, further comprising deactivating the cleavage agent after cleaving the polypeptide.

60. (Previously Presented) The method according to claim 17, further comprising reducing cysteine disulphide bridges in the polypeptide to form free thiols and capping the free thiols, prior to cleaving the polypeptide.

61. (Previously Presented) The method according to claim 17, further comprising deactivating the cleavage agent after cleaving the polypeptide.

62. (Previously Presented) The method according to claim 19, further comprising reducing cysteine disulphide bridges in the polypeptide to form free thiols and capping the free thiols, prior to capping one or more ϵ -amino groups.

63. (Previously Presented) The method according to claim 19, further comprising deactivating the cleavage agent after cleaving the polypeptides.

64. – 70. (Canceled).